Structure and conserved function of iso-branched sphingoid bases from the nematode *Caenorhabditis elegans*

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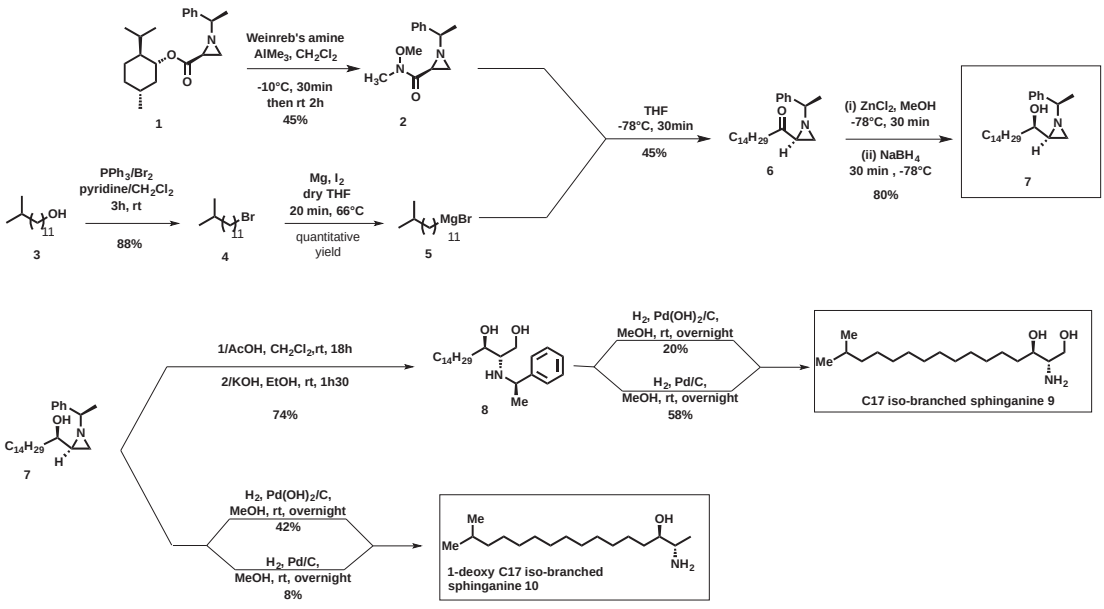
Supplementary Information on Chemical Synthesis

General scheme:

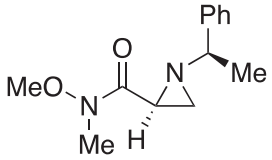


Experimental Part:

General reagents and materials

12-methyl tridecanol was from Endeavour Speciality Chemicals, all other starting compounds and solvents were purchased from Sigma-Aldrich/Fluka or Acros and were used without further purification. Column chromatographic separations were carried out using 230-400 mesh silica gel. TLC plates were developed with potassium permanganate mixture (1 g of $KMnO_4$, 2 g of Na_2CO_3, 100 mL of H_2O). ^1H, and ^{13}C NMR spectra were recorded (as indicated) on either a Bruker 300 MHz or 400 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s) or triplet (t) with coupling constants (J) given in Hz, or multiplet (m). ESI-MS for the characterization of compounds was performed on an ESI API 150EX and are reported as mass-per-charge ratio. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate). Optical rotation was measured on a Jasco P-1030 polarimeter. All reactions were performed under an Ar or N_2 atmosphere.

(*S*)-*N*-methoxy-*N*-methyl-1-((*R*)-1-phenylethyl)aziridine-2-carboxamide (2)



A literature protocol was followed.[1]

Method A : To (2*S*)-2-isopropyl-4-methoxycyclohexyl 1-((*R*)-1-phenylethyl)aziridine-2-carboxylate **1** (1.00 g, 2.89 mmol, 1 equiv.) and Weinreb's amine or *N*,*O*-dimethylhydroxylamine.HCl (450 mg, 7.4 mmol, 2.5 equiv.) in 10 mL THF was slowly added isopropyl magnesium chloride (4.60 mL, 9.2 mmol, 3.2 equiv., 2.0 M in THF) at 0 °C. The resulting mixture was warmed to rt and after 30 min, it was partitioned between CH_2Cl_2 and H_2O. The water phase was washed with CH_2Cl_2 (3 × 20 mL). The combined organic solvents were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified on a silica gel column (EtOAc/Pentane gradient 1:1 to 1:0) in order to give Weinreb's amide or *N*-(*R*)-(+)-α-methylbenzyl-2-(*S*)-aziridine *N*-Methoxy-*N*-methylcarboxamide **2** as a white powder (298 mg, 44 %).

Method B : To the solid of *N*,*O*-dimethylhydroxylamine·HCl (880 mg, 9.11 mmol, 3 equiv.) in 10 mL of CH_2Cl_2 was carefully added trimethylaluminium (4.55 mL, 9.11 mmol, 3 equiv., 2.00M) under nitrogen at -10 °C. The solution was stirred for 30 min at rt and then a solution of (2*S*)-2-isopropyl-4-methoxycyclohexyl 1-(*R*-1-phenylethyl)aziridine-2-carboxylate **1** (1.00 g, 3.04 mmol) in 5.0 mL CH_2Cl_2 was added dropwise at -10 °C. The mixture was stirred for 2 h at rt. Then the reaction was quenched carefully with water and the organic layer was separated. After extraction with CH_2Cl_2 (3 × 20 mL), the combined organic layers were dried, filtered, and concentrated under vacuum. Purification by silica gel flash chromatography (EtOAc/cyclohexane, 75:15 to 50:50 to 100:0) yielded pure *N*-(*R*)-(+)-α-methylbenzyl-2-(*S*)-aziridine *N*-Methoxy-*N*-methylcarboxamide **2** as an oil (321 mg, 45%).

$[\alpha]^{22}_D$ = + 13.1 (c 1.00, $CHCl_3$).[2]

R_f=0.10 (EtOAc/Pentane 1:0).

^1H NMR (300 MHz, $CDCl_3$) : δ = 7.44 – 7.08 (m, 5H) , 3.11 (d, *J* = 8.3 Hz, 6H), 2.55 (q, *J* = 6.4 Hz, 2H), 2.40 (d, J = 3.0 Hz, 1H), 1.74 (d, *J* = 6.4 Hz, 1H), 1.46 (d, *J* = 6.5 Hz, 3H).

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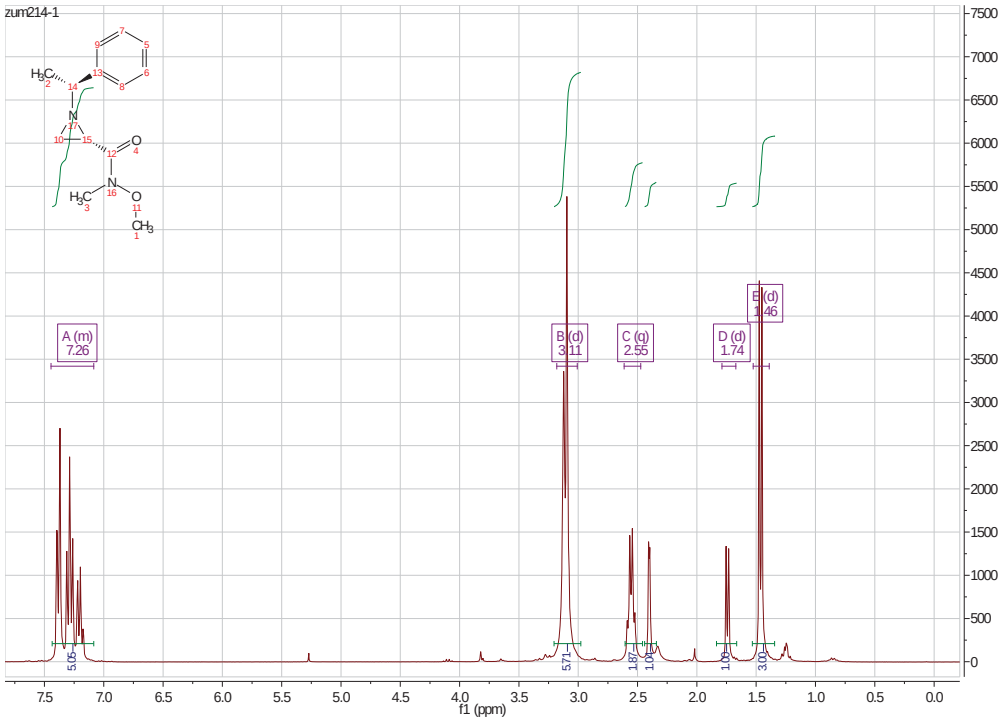
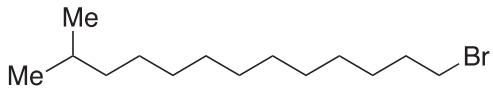
[1] J-W. Kim, Y-W. Kim, Y. Inagaki, Y-A. Hwang, S. Mitsutake, Y-W. Ryu, W. K. Lee, H-J. Ha, C-S. Park, Y.Igarashi, *Bioorganic & Medicinal Chemistry*, **2005**, *13*, 3475-3485.

[2] This value has been confirmed by personal communication with Ha *et al*. The reported value in *J. Org. Chem*. **2003**, *68*, 7675-7680 stands corrected.



1-bromo-12-methyltridecane (4)



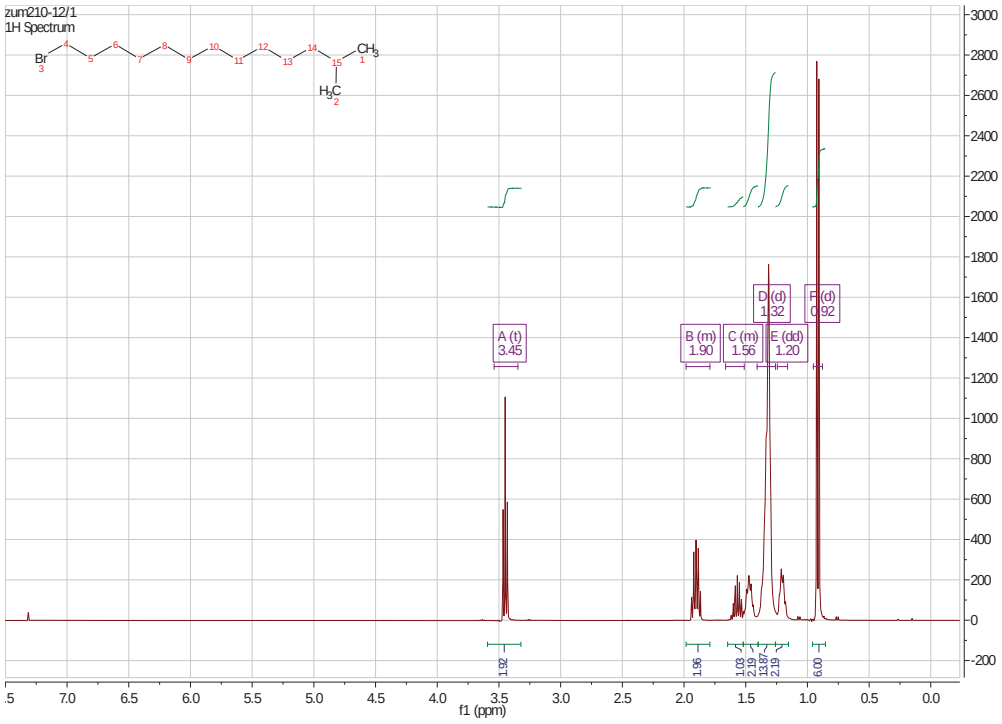
Method A : 12-methyl-1-tridecanol **3** (1.00 g, 4.66 mmol) was dissolved in CH_3CN and bromotrimethylsilane (1.79 g, 11.7 mmol, 2.5 equiv.) was added. The solution was heated to 90 °C for 5 h. Then 2 mL H_2O were added. The solvents were removed under reduced pressure and the crude material was purified on a silica gel column (hexanes) in order to give 1-bromo-12-methyltridecane as a clear oil (380 mg, 29 %).

Method B : 12-methyl-1-tridecanol **3** (2.00 g, 9.3 mmol) was dissolved in CH_2Cl_2 (25 mL) and PPh_3 (4.40 g, 16.6 mmol, 1.2 equiv.), and pyridine (1.3 mL, 16.6 mmol, 1.2 equiv.) were added. The flask was cooled down to 0 °C after that bromine Br_2 was added dropwise (0.56 mL, 16.6 mmol, 1.2 equiv.). The solution became orange and the reaction was stirred for 3 h at rt. The solvent was removed under reduced pressure and the orange crude material was filtered on a silica gel column using CH_2Cl_2 to afford to 1-bromo-12-methyltridecane **4** as yellow oil (3.42 g, 88 %).

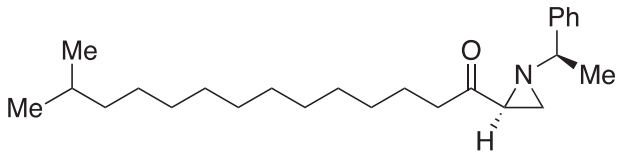
R_f = 0.75 (Hexanes).

^{1}H NMR[3] (400 MHz, $CDCl_3$) : δ = 3.45 (t, *J* = 6.9 Hz, 2H), 1.98 – 1.79 (m, 2H), 1.66 – 1.51 (m, 1H), 1.47 (dd, *J* = 14.3, 7.0 Hz, 2H), 1.32 (d, *J* = 7.3 Hz, 14H), 1.20 (dd, *J* = 13.3, 6.5 Hz, 2H), 0.92 (d, *J* = 6.6 Hz, 6H).

[3] J.Y. Mun, A. Onorato, F. C. Nichols, M. D. Morton, A. I. Saleh, M. Welzel, M. B. Smith. *Organic & Biomolecular Chemistry*, **2007**, 5, 3826-3833.



13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-one (6)



1-bromo-12-methyltridecane **4** (413 mg, 1.7 mmol, 1.5 equiv.) was dissolved in 2 mL THF. Mg turnings (40.3 mg, 1.7 mmol, 1.5 equiv.) were added together with a drop of I_2. The reaction was heated in order to initiate the Grignard reaction. The mixture of **5** was slowly added to (*S*)-*N*-methoxy-*N*-methyl-1-((*R*)-1-phenylethyl)aziridine-2-carboxamide **2** (259 mg, 1.1 mmol) in 5 mL THF at -78 °C. The reaction was stirred for 30 min and then the cold-bath was removed. After reaching rt, the mixture was quenched with 5 mL H_2O and extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried over $MgSO_4$, filtered, and the solvents were removed under reduced pressure. The crude material was purified on a silica gel column (EtOAc/Pentane 1:4) and the pure compound **6** was isolated as a clear oil (199 mg, 0.54 mmol, 48 %).

$[\alpha]^{24}_D$ = - 53.9 (c 1.00, $CHCl_3$).

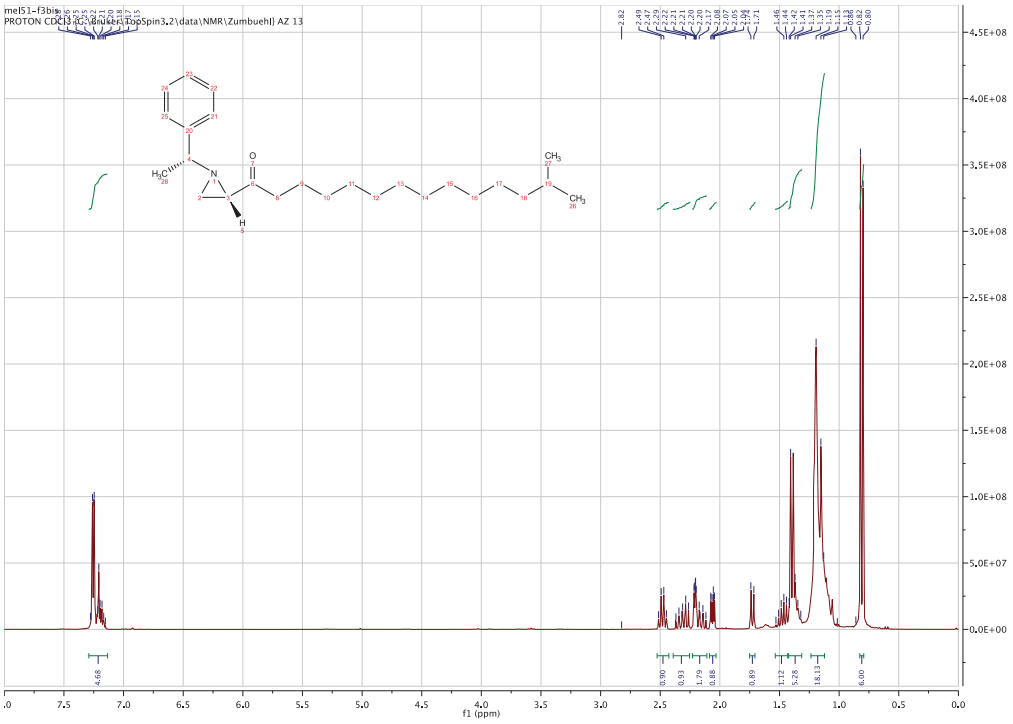
R_f = 0.40 (EtOAc:Pentane 1:9).

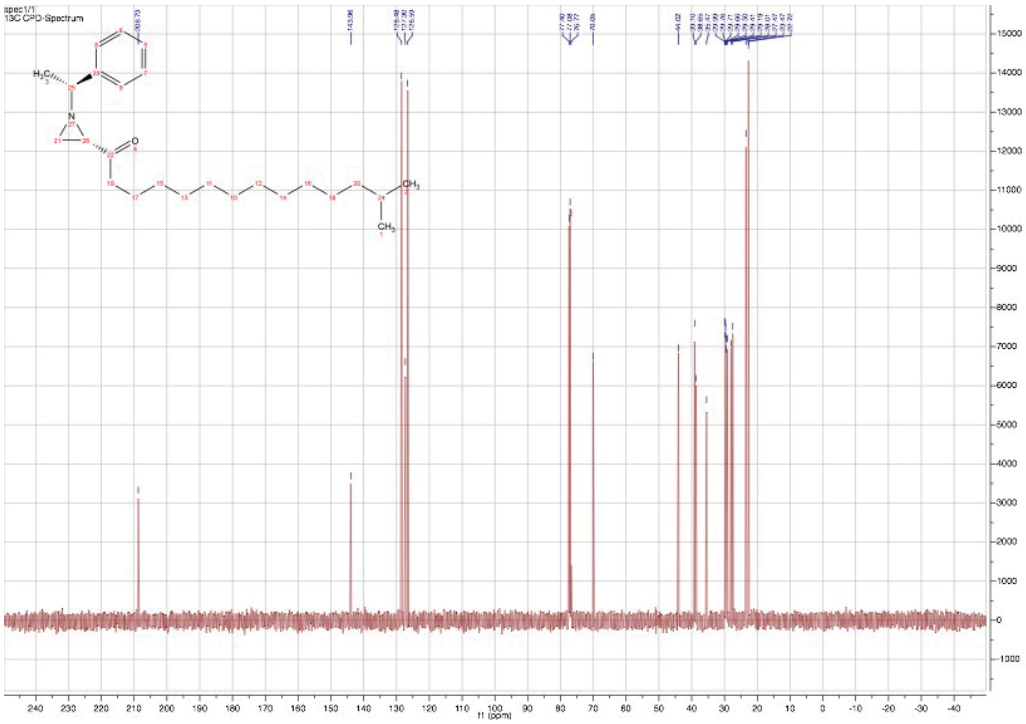
HRMS-ESI (m/z) : $[M+H]^+$ calcd for $C_{25}H_{42}NO$: 372.326; found 372.3259.

IR (Golden Gate) : 2925 (s), 2853 (m), 1702 (m), 1451 (w).

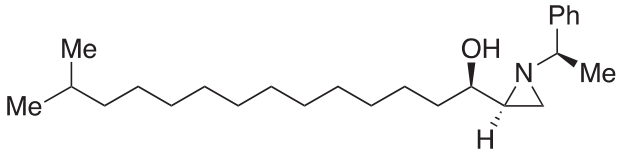
^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.28 – 7.15 (m, 5H), 2.48 (q, *J* = 6.6 Hz, 1H), 2.37 – 2.25 (m, 1H), 2.23 – 2.11 (m, 2H), 2.06 (dd, *J* = 6.7, 3.1 Hz, 1H), 1.73 (d, *J* = 6.8 Hz, 1H), 1.53 – 1.43 (m, 1H), 1.42 – 1.31 (m, 5H), 1.26 – 1.02 (m, 18H), 0.81 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) : δ = 208.73, 143.96, 128.48, 127.30, 126.59, 70.05, 44.02, 39.10, 38.65, 35.47, 29.99, 29.76, 29.71, 29.66, 29.50, 29.41, 29.19, 28.01, 27.47, 23.47, 22.72.





(*R*)-13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-ol (7)



Method A : To 13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-one **6** (150 mg, 404 µmol) in 2 mL dry MeOH at - 78 °C, was added $ZnCl_2$ (81.0 mg, 594 µmol, 1.47 equiv.). After 30 min. $NaBH_4$ (29.5 mg, 780 µmol, 1.9 equiv.) was added and the mixture was stirred for additional 30 min. Then, 5 mL H_2O was added at -78 °C and the reaction was left to reach rt. The water phase was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude material was purified on a silica gel column (EtOAc/Hexanes 3:7) and the pure compound **7** was isolated (95.0 mg, 254 µmol, 63 %).

Method B : To 13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-one **6** (55 mg, 148 µmol) in 2 mL dry MeOH –t - 78 °C was added $ZnCl_2$ (29.7 mg, 217.8 µmol). After 30 min. $NaBH_4$ (10.8 mg, 285.5 µmol, 1.47 equiv.) was added and the mixture was stirred for additional 30 min. Then, H_2O (1 mL) was added at -78 °C and the reaction was left to reach rt. The water phase was extracted 3 times with CH_2Cl_2. The organic solvent was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude material **7** (44 mg, crude yield : 80 %) was directly used in the followed step.

$[\alpha]^{24}_D$ = + 7.0 (c 0.50, $CHCl_3$).

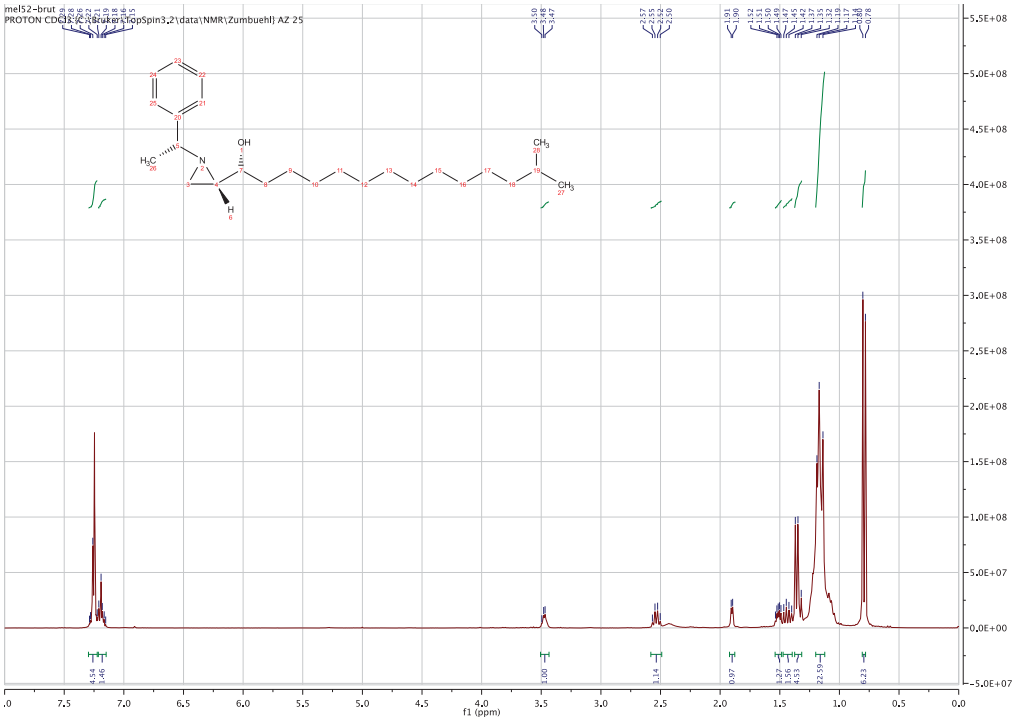
R_f = 0.10 (EtOAc/Hexanes 3:7).

HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{44}NO$: 374.3417; found 374.3413.

IR (Golden Gate): 2926 (s), 2851 (m), 1703 (m), 1371 (m), 1287 (m).

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.30 – 7.23 (m, 4H), 7.22 – 7.13 (m, 1H), 3.52 – 3.41 (m, 1H), 2.53 (q, *J* = 6.5 Hz, 1H), 1.92 (d, *J* = 3.4 Hz, 1H), 1.55 – 1.48 (m, 1H), 1.47 – 1.39 (m, 1H), 1.38 – 1.30 (m, 4H), 1.26 – 1.01 (m, 22H), 0.81 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) : δ = 144.35, 128.47, 127.20, 126.64, 99.99, 69.36, 68.34, 42.17, 39.10, 34.71, 29.99, 29.77, 29.72, 29.63, 29.57, 28.01, 27.47, 25.27, 23.15, 22.72.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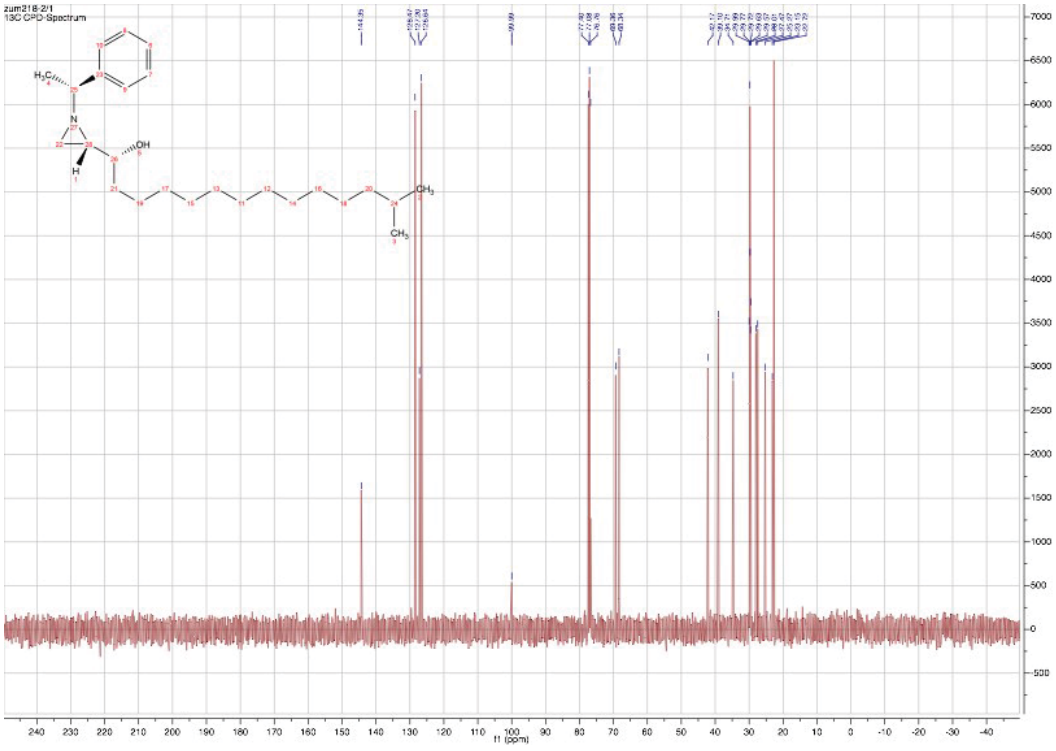
f1 (ppm)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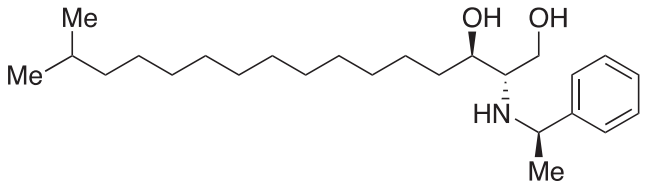
f1 (ppm)

(2*S*,3*R*)-15-methyl-2-(((*R*)-1-phenylethyl)amino)hexadecane-1,3-diol (8)



Method A : To (*R*)-13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-ol **7** (82.2 mg, 220 µmol) in 2 mL of CH_2Cl_2 was added acetic acid (66.0 µL, 1.10 mmol, 5 equiv.) and the reaction was stirred for 18 h. Then 1 mL of $NaHCO_3$ (sat.) was added and the solution was extracted with CH_2Cl_2 (4 × 10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude material was purified over a short silica gel column (EtOAc) to give pure compound **8** (28.3 mg, 72.2 µmol, 33 %).

Method B : To (*R*)-13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)tetradecan-1-ol **7** (25 mg, 66 µmol) in 0.8 mL of CH_2Cl_2 was added acetic acid (20.0 µL, 0.33 mmol, 5 equiv.) and the reaction was stirred for 2 days. Then 16 µL of acetic acid were added and the mixture was stirred for 2 h. Then 5 mL of $NaHCO_3$ (sat.) was added and the solution was extracted with CH_2Cl_2 (3 × 10 mL) and brine (3 × 10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product (clear oil, colorless) was purified over a short silica gel column (EtOAc), dissolved in 5 mL of CH_2Cl_2 and 20 µL of acetic acid were added. After 18 h, the reaction was quenched with 1 mL of $NaHCO_3$ and the solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. Then, the product was dissolved in 0.5 mL of EtOH and 4.2 mg (75 mmol) of KOH were added. After stirring for 1.5 h the solvent was evaporated under reduced pressure. After the addition of H_2O, the solution was extracted with CH_2Cl_2 (3 × 10 mL), the organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. A short silica gel column (EtOAc) afforded compound **8** (19 mg, 50 µmol) in 74 % yield.

$[\alpha]^{24}_D$ = + 21.5 (c 1.00, $CHCl_3$).

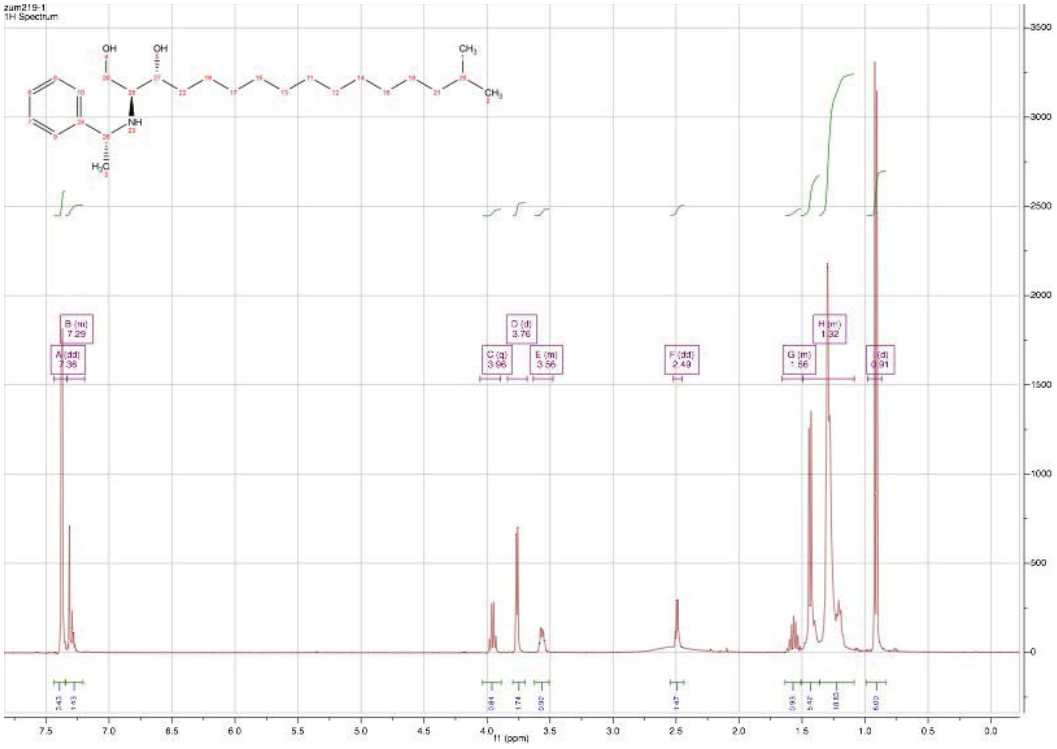
R_f = 0.19 (EtOAc).

HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{46}NO_2$: 392.3523; found 392.3526.

IR (Golden Gate): 3349 (br), 2923 (s), 2852 (m), 1466 (m), 1366 (s), 1065 (m).

^{1}H NMR (400 MHz, $CDCl_3$) : δ = 7.36 (dd, *J* = 10.2, 2.8 Hz, 4H), 7.33 – 7.19 (m, 1H), 3.96 (q, *J* = 6.5 Hz, 1H), 3.76 (d, *J* = 4.1 Hz, 2H), 3.63 – 3.52 (m, 1H), 2.49 (dd, *J* = 8.0, 4.0 Hz, 2H), 1.62 – 1.52 (m, 1H), 1.48 – 1.40 (m, 6H), 1.35 – 1.18 (m, 20H), 0.91 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) : δ = 145.64, 128.59, 127.18, 126.62, 73.59, 60.28, 58.92, 55.94, 39.10, 33.80, 29.99, 29.77, 29.72, 29.69, 29.63, 29.59, 29.56, 28.01, 27.47, 26.12, 24.60, 22.71.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A (dd)
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D (d)
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C (q)
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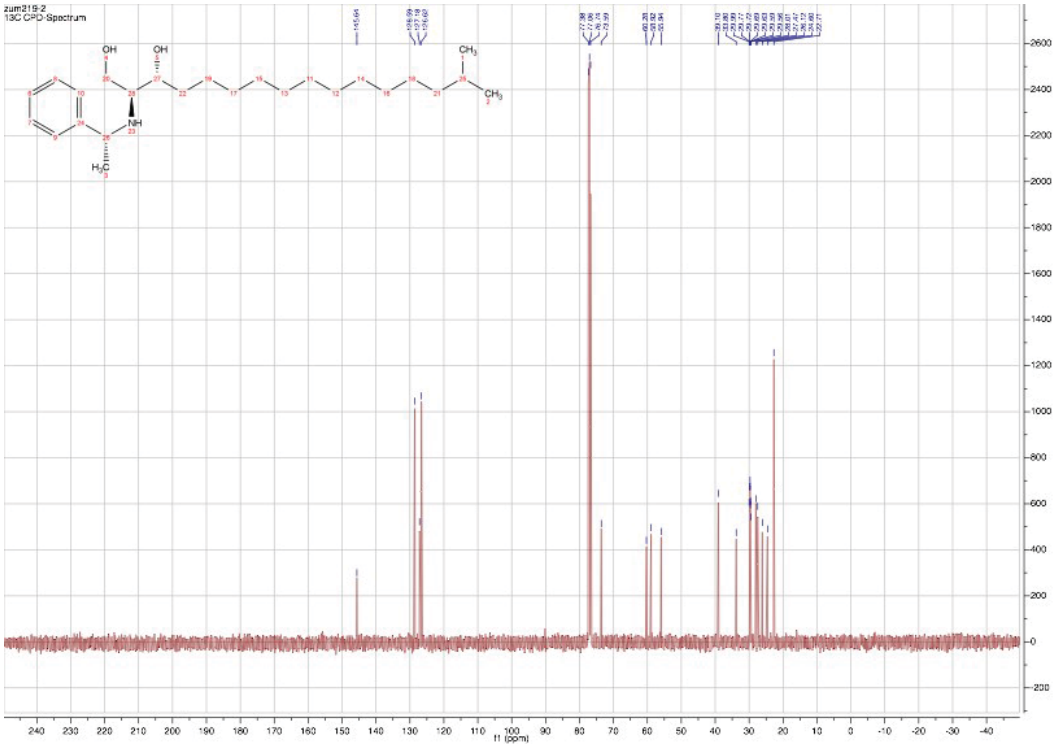
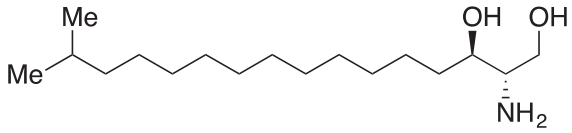
H (m)
1.32
G (m)
1.56
I (d)
0.91
f1 (ppm)


zum219-2
13C CPD-Spectrum
f1 (ppm)

(2*S*,3*R*)-2-amino-15-methylhexadecane-1,3-diol (9)



Method A : To (2*S*,3*R*)-15-methyl-2-(((*R*)-1-phenylethyl)amino)hexadecane-1,3-diol **8** (28.3 mg, 72 µmol) in 5 mL MeOH was added $Pd(OH)_2/C$ (5 mg) and the mixture was stirred under 1 atm. of H_2 for 48 h. Then the mixture was filtered and the solvents were evaporated under reduced pressure. The crude material was purified on a silica gel column (CH_2Cl_2/MeOH/NH_4OH 0.875:0.11:0.015) to give compound **9** (4.20 mg, 14.6 µmol) in 20 % yield.

Method B : To (2*S*,3*R*)-15-methyl-2-(((*R*)-1-phenylethyl)amino)hexadecane-1,3-diol **8** (27 mg, 48 µmol) in 5 mL MeOH was added Pd/C (10 mg) and the mixture was stirred under 1 atm. of H_2 overnight. Then the mixture was filtered over celite and the solvent was evaporated under reduced pressure to afford to the compound **9** (8 mg, 27.8 µmol) in 58 % yield.

$[\alpha]^{22}_D$ = - 4.4 (c 0.33, $CHCl_3$).

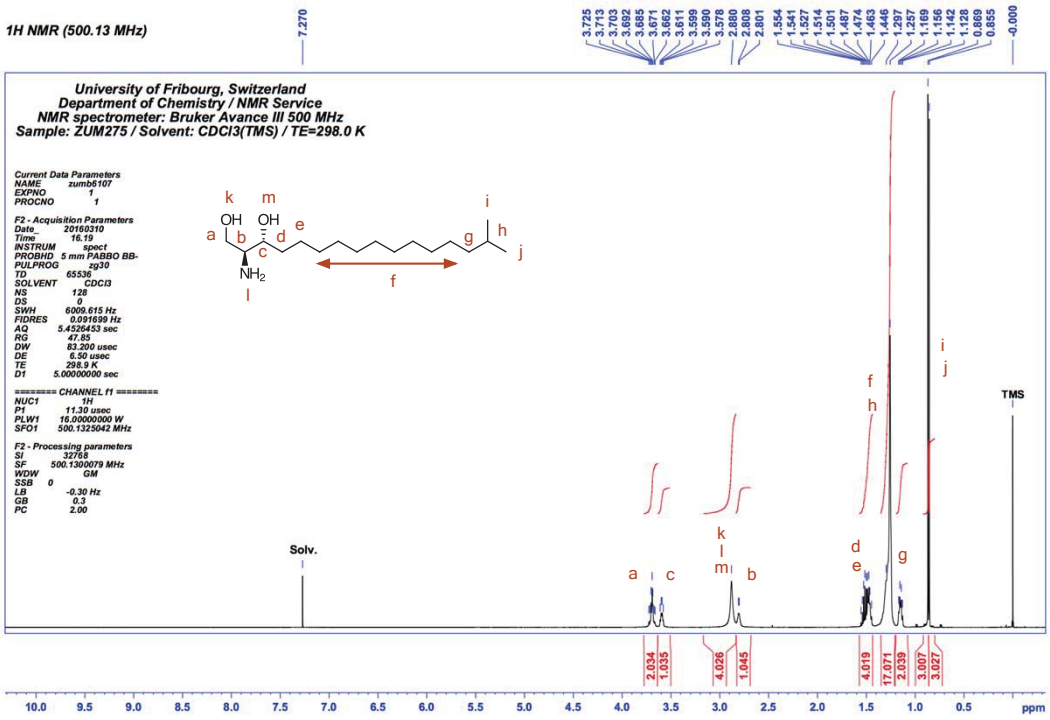
R_f = 0.27 (CH_2Cl_2/MeOH/NH_4OH 0.875:0.11:0.015).

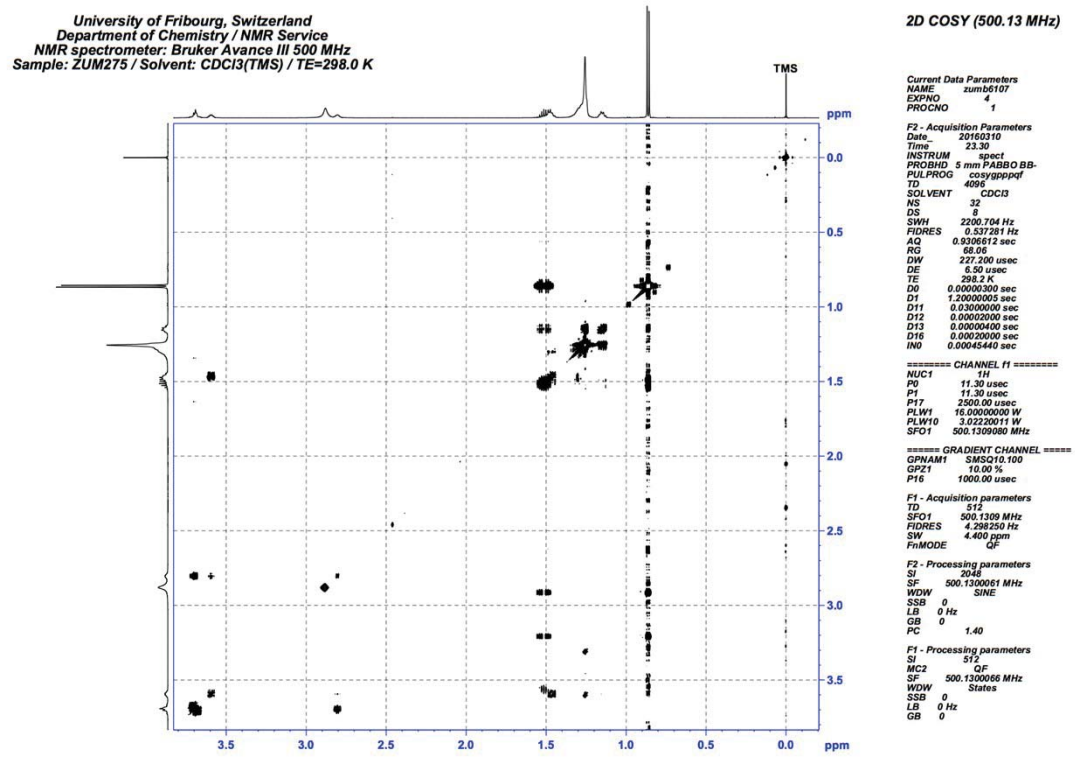
HRMS-ESI (m/z) : $[M+H]^+$ calcd for $C_{17}H_{38}NO_2$: 288.2897; found 288.2892.

IR (Golden Gate) : 3339 (br), 2922 (s), 2852 (m), 1467 (m), 1384 (w), 1366 (w), 1215 (w), 1051 (w).

^{1}H NMR (500 MHz, $CDCl_3$) : δ = 3.73 – 3.67 (m, 2H), 3.66 – 3.58 (m, 1H), 2.88 (br, OH, OH, NH_2, 4H), 2.81 – 2.80 (m, 1H), 1.55 – 1.45 (m, 4H), 1.30 – 1.26 (m, 17H), 1.15 (dt, *J* = 7.3, 6.5 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) : δ = 74.48, 63.34, 55.77, 39.08, 33.84, 29.97, 29.76, 29.74, 29.72, 29.71, 29.68, 27.98, 27.44, 26.14, 22.67.




University of Fribourg, Switzerland
Department of Chemistry / NMR Service
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TMS
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Current Data Parameters
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PROCNO 1
F2 - Acquisition Parameters
Date_ 20160310
Time 23.30
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PROBHD 5 mm PABBO BB-
PULPROG cosygpppqf
TD 4096
SOLVENT CDCl3
NS 32
DS 8
SWH 2200.704 Hz
FIDRES 0.537281 Hz
AQ 0.9306612 sec
RG 68.06
DW 227.200 usec
DE 6.50 usec
TE 298.2 K
D0 0.00000300 sec
D1 1.20000005 sec
D11 0.03000000 sec
D12 0.00002000 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00045440 sec
======== CHANNEL f1 ========
NUC1 1H
P0 11.30 usec
P1 11.30 usec
P17 2500.00 usec
PLW1 16.00000000 W
PLW10 3.02220011 W
SFO1 500.1309080 MHz
====== GRADIENT CHANNEL =====
GPNAM1 SMSQ10.100
GPZ1 10.00 %
P16 1000.00 usec
F1 - Acquisition parameters
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SFO1 500.1309 MHz
FIDRES 4.298250 Hz
SW 4.400 ppm
FnMODE QF
F2 - Processing parameters
SI 2048
SF 500.1300061 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
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SI 512
MC2 QF
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WDW States
SSB 0
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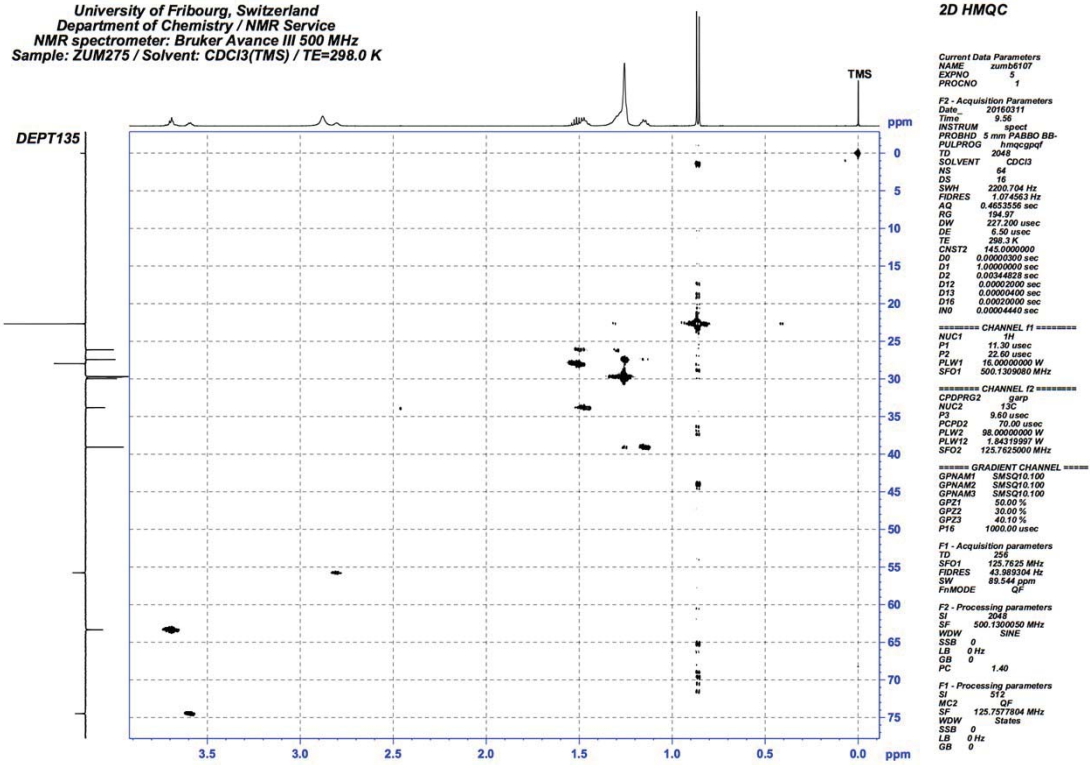
University of Fribourg, Switzerland
Department of Chemistry / NMR Service
NMR spectrometer: Bruker Avance III 500 MHz
Sample: ZUM275 / Solvent: CDCl3(TMS) / TE=298.0 K
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DEPT135
TMS
ppm
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PROCNO 1
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Time 9.56
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DE 6.50 usec
TE 298.3 K
CNST2 145.0000000
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D2 0.00344828 sec
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NUC1 1H
P1 11.30 usec
P2 22.60 usec
PLW1 16.00000000 W
SFO1 500.1309080 MHz
======== CHANNEL f2 ========
CPDPRG2 garp
NUC2 13C
P3 9.60 usec
PCPD2 70.00 usec
PLW2 98.00000000 W
PLW12 1.84319997 W
SFO2 125.7625000 MHz
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GPNAM2 SMSQ10.100
GPNAM3 SMSQ10.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec
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FIDRES 43.989304 Hz
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F2 - Processing parameters
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WDW SINE
SSB 0
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WDW States
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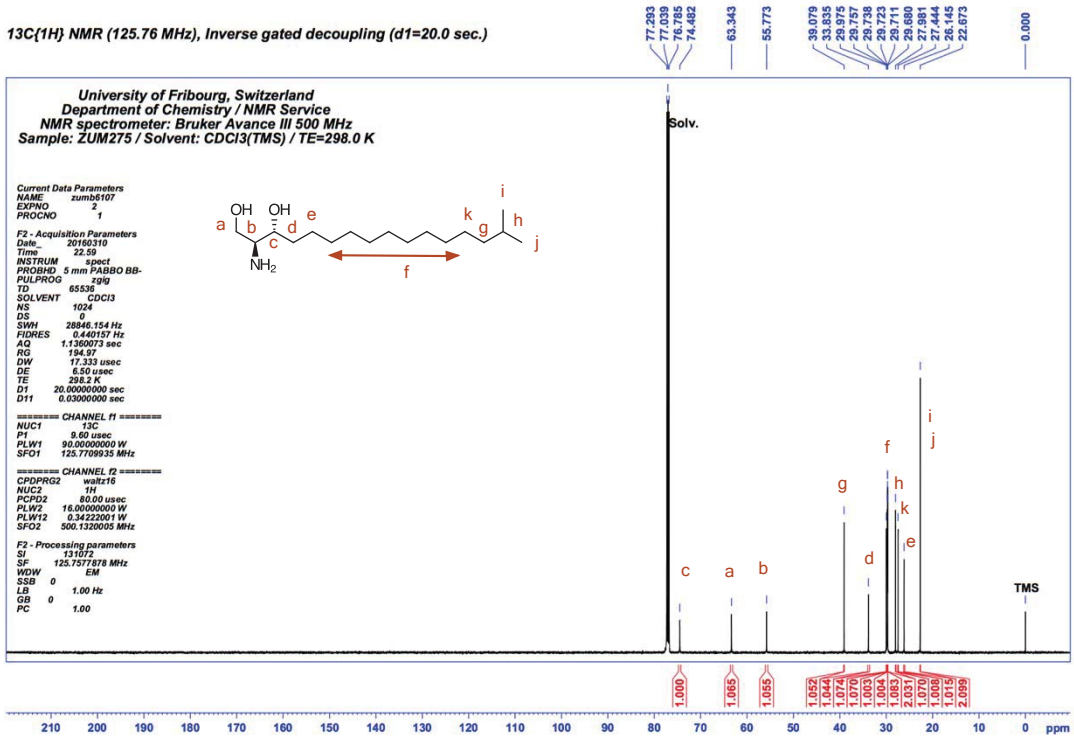
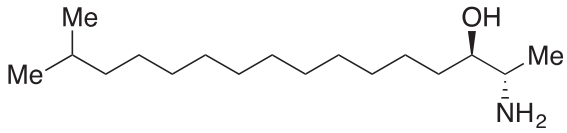


(2*S*,3*R*)-2-amino-15-methylhexadecan-3-ol (10)



Method A : To (*R*)-13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl) tetradecan-1-ol **7**, (25 mg, 150 µmol) in 1.8 mL MeOH was added Pd/C (1.3 mg) and the mixture was stirred under 1 atm. of H_2 for 48 h. Then the mixture was filtered and the solvent was evaporated under reduced pressure. The crude material was purified on a silica gel column (CH_2Cl_2/MeOH/NH_4OH 0.875:0.11:0.015) to give compound **9** (3 mg, 11 µmol, 8 %).

Method B : To (*R*)-13-methyl-1-((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl) tetradecan-1-ol **7** (65.9 mg, 176 µmol) in 5 mL MeOH was added $Pd(OH)_2/C$ (7 mg,) and the mixture was stirred under 1 atm. of H_2 for 48 h. Then the mixture was filtered and the solvents were evaporated under reduced pressure. The crude material was purified on a silica gel column (CH_2Cl_2/MeOH/NH_4OH 0.875:0.11:0.015) to give compound **10** (20.2 mg, 74.4 µmol, 42 %).

$[\alpha]^{24}_D$ = + 8.6 (c 1.00, $CHCl_3$).

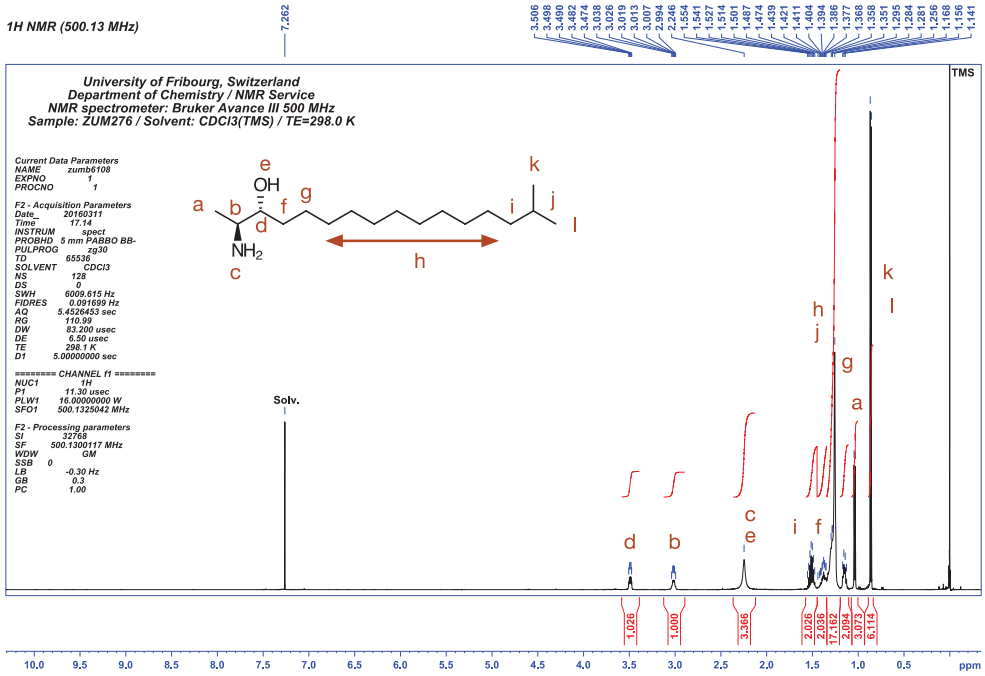
R_f = 0.15 (CH_2Cl_2/MeOH/NH_4OH 0.875:0.11:0.015).

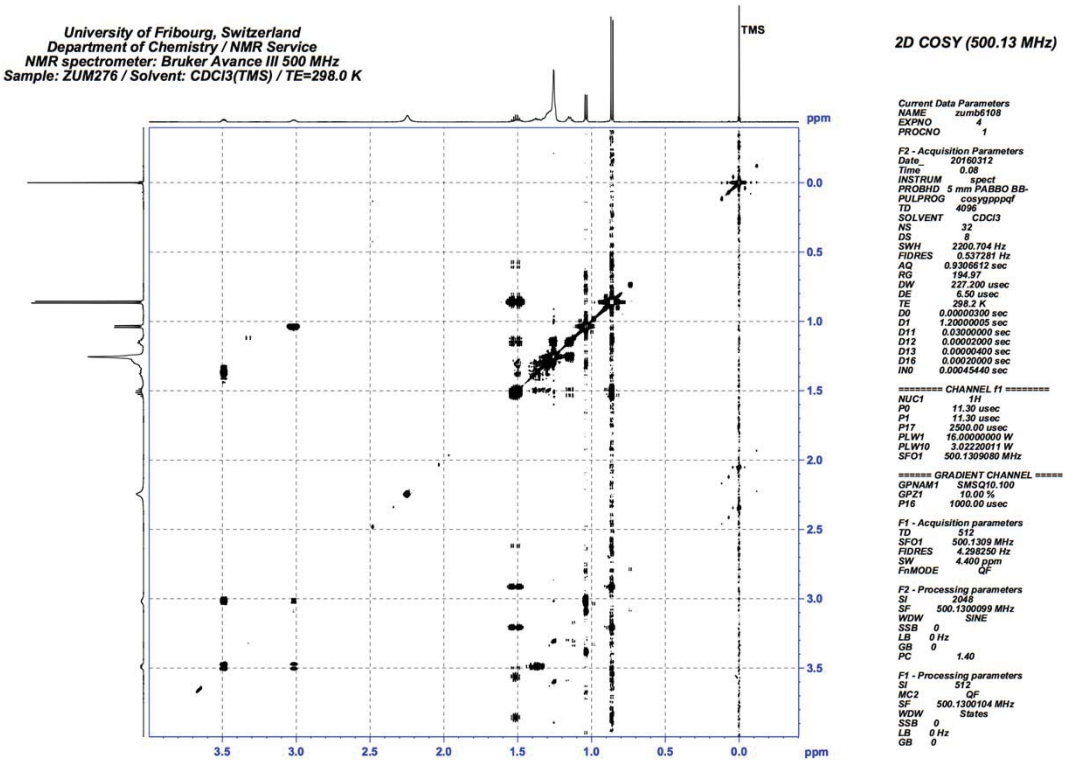
HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{17}H_{38}NO$: 272.2947; found 272.2950.

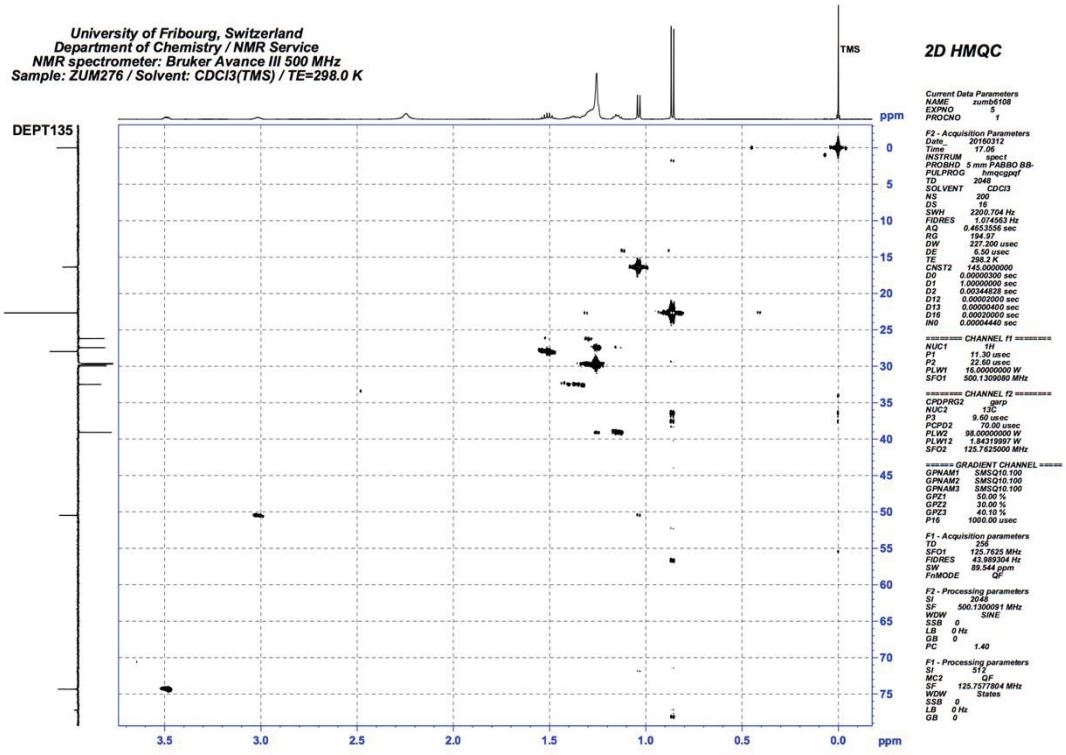
IR (Golden Gate): 2915 (s), 2850 (s), 1587 (m), 1468 (m), 1366 (m), 1093 (m).

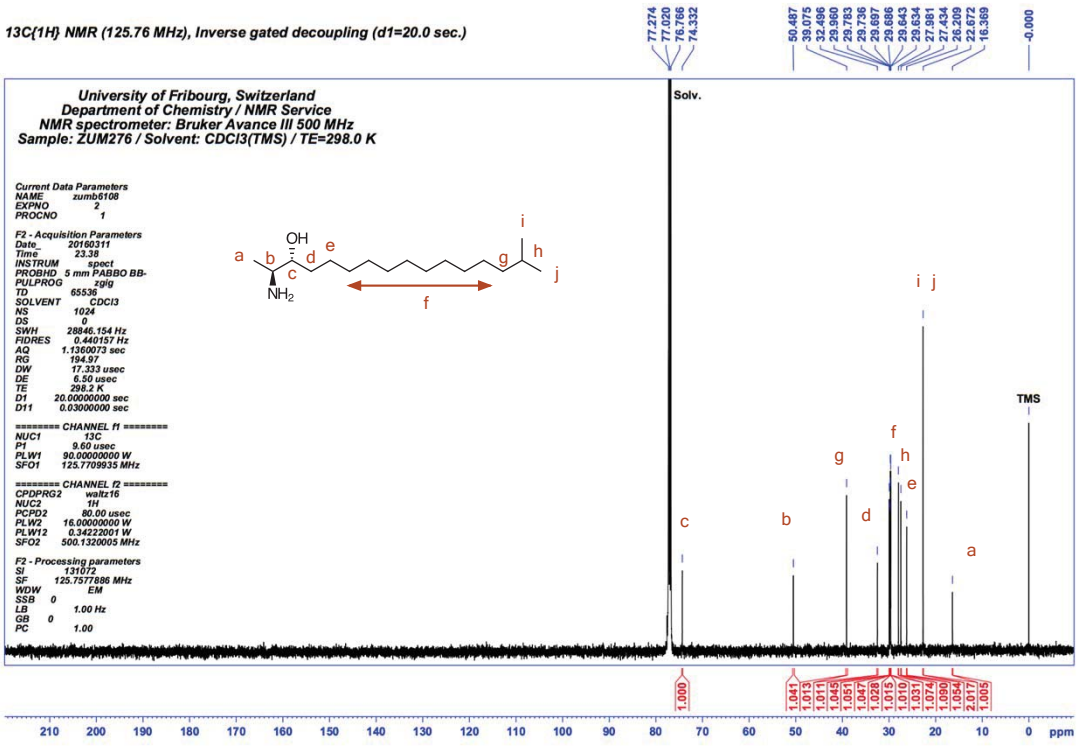
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (dt, *J* = 4.2, 3.8 Hz, 1H), 3.01 (dq, *J* = 6.2, 3.3, 3.2 Hz, 1H), 2.25 (br, OH/NH_2, 3H), 1.55 – 1.47 (m, 2H), 1.44 – 1.35 (m, 2H), 1.30 – 1.26 (m, 17H), 1.17 – 1.13 (m, 2H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (125.76 MHz, $CDCl_3$) δ 74.33, 50.49, 39.07, 32.50, 29.96, 29.78, 29.74, 29.67, 29.69, 29.64, 29.63, 27, 98, 27.43, 26.21, 22.67, 16.37.





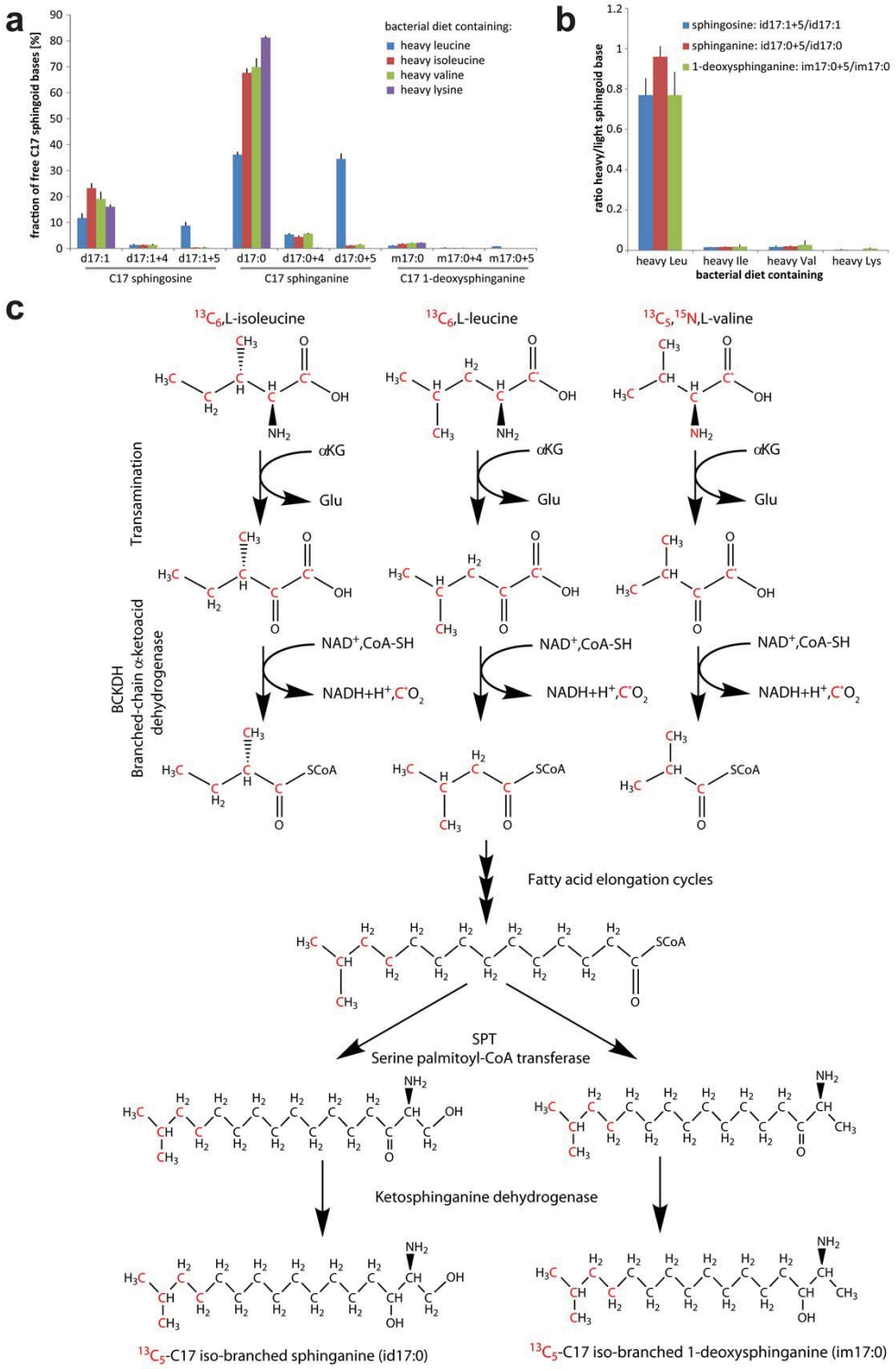

University of Fribourg, Switzerland
Department of Chemistry / NMR Service
NMR spectrometer: Bruker Avance III 500 MHz
Sample: ZUM276 / Solvent: CDCl3(TMS) / TE=298.0 K
2D HMQC
DEPT135
TMS
ppm


13C{1H} NMR (125.76 MHz), Inverse gated decoupling (d1=20.0 sec.)
University of Fribourg, Switzerland
Department of Chemistry / NMR Service
NMR spectrometer: Bruker Avance III 500 MHz
Sample: ZUM276 / Solvent: CDCl3(TMS) / TE=298.0 K
Solv.
TMS
ppm

Supplementary Figures:

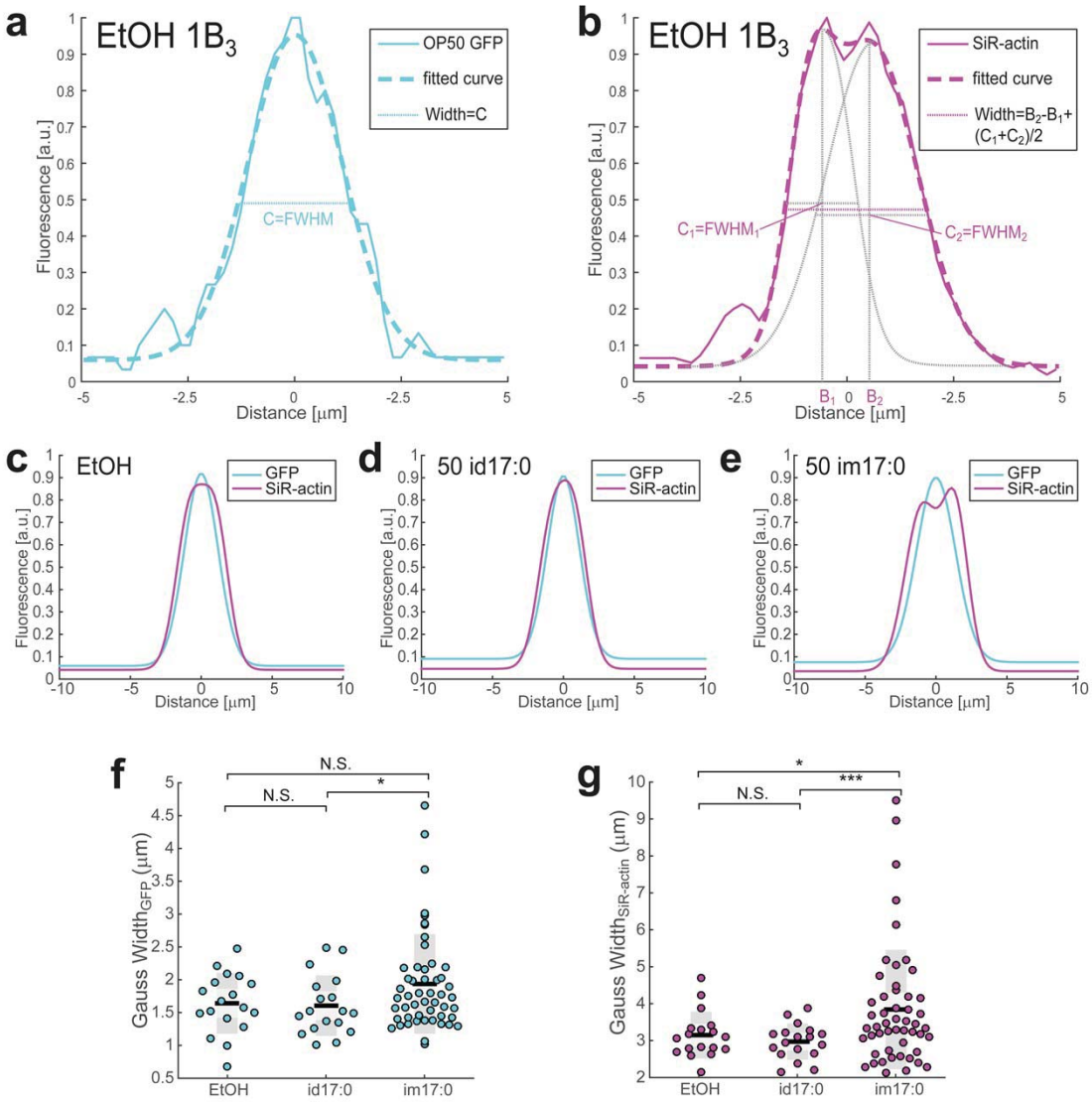
Supplementary Figure 1.

Labelling of *C. elegans* sphingoid bases using amino acids. **(a)** C17 sphingoid bases detected in animals fed with bacterial diets containing heavy amino acids leucine, isoleucine, valine or lysine, the light and the ^{13}C-containing +4 and +5 isotopic peaks are shown; **(b)** relative amounts of +5 isotopic peak with 13C label incorporation compared to light C17 sphingoid base without isotopic peak correction; **(c)** scheme of label incorporation from heavy isotope labelled amino acids via branched chain fatty acids into sphingoid bases.



Supplementary Figure 2.

Calculation of width of GFP positive intestinal lumen and width of F-actin signal visualized by SiR-actin. **(a-b)** representative line profile of fluorescent signals from OP50 GFP in the intestinal lumen (cyan line, **a**) and SiR-actin (magenta line, **b**) and their Gaussian fits (dashed lines) recorded perpendicular to the intestinal lumen in animals treated with ethanol vehicle; **(c)-(e)** average Gaussian fits of GFP signals (cyan) and SiR-actin (magenta) for animals treated with solvent control EtOH **(c)**, C17 iso-branched sphinganine, id17:0 **(d)** and 1-deoxy C17 iso-branched sphinganine, im17:0 **(e); (f)** width of the intestinal lumen as given by the full width at half maximum of a single Gaussian fit of the GFP signal; **(g)** width of the apical F-actin signal as calculated by the width of two Gaussian fits of the SiR-actin signal, statistical significance determined by Welch's t-test * $p<0.05$, *** $p<0.005$.



Supplementary Figure 3.

Saturated image of Figure 5 to better visualize growth of *lcb1*Δ strain on racemic C16 DL-sphinganine (DL-d16:0).